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# Electronic Supplementary Information

## Phospholipid-stabilized $Cu_xAg_{1-x}InSe_2$ Nanocrystals as luminophores: Fabrication, Optical Properties and Biological Application

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## *In vitro* experiment

**Cell culture**

Human breast cancer MCF-7 cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS, GIBCO, Waltham, MA, USA). Human hepatoma Hep G2 cells were cultured in Eagle's Minimum Essential Medium, supplemented with 10% FBS, 1% L-glutamine and 1% antibiotic antimycotic formulation. Cells were incubated in a 5 % $CO_2$ incubator at 37 °C.

CCSCs were sorted from SW-620 cells using CD24 marker, and further identified using CD133 by immunohistochemistry. Cells were cultured in serum-free culture medium in a low-adherence culture flask containing epidermal growth factor and leukaemia inhibitory factor, supplemented with 1% antibiotic antimycotic formulation. Cells were incubated in a 5 % $CO_2$ incubator at 37 °C.

**MTT assay**

MCF-7 cells were seeded at a density of $8\times10^3$ cells per well into 96-well plates. After 24 h incubation, cells were treated with various concentrations of NPs for 24 h. Then 20 $\mu$L MTT (5 mg/mL) was added to each well and incubated for another 4 h. The resulting formazan crystals were dissolved using 150 $\mu$L dimethyl sulfoxide (DMSO). Absorbance value at 490 nm was then measured using a microplate reader.

**Flow cytometry analysis**

The effects of NPs on apoptosis were determined by flow cytometry using an Annexin V-FITC/PI kit (Vazyme, Nanjing, China). Briefly, the cells were harvested at 24 h after NPs treatment and then cells were washed twice with cold PBS. Cells were resuspended in 100 $\mu$L binding buffer and stained with 5 $\mu$L Annexin V-FITC and 5 μL PI in the dark for 15 min. Then, 400 μL binding buffer were added to stop the reaction. The stained cells were analyzed by flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

**Fluorescence Imaging**

Cells were seeded in 24-well plate at a density of $3\times10^4$ cells per well and cultured overnight. 220 $\mu$L of DMPC-NCs dispersion was added into the cells and incubated at 37 °C for a set time. After washing with PBS three times, the cells were incubated with (4',6-diamidino-2-phenylindole dihydrochloride) DAPI for 5 min for nucleus staining. The fluorescence images were recorded with an inverted confocal laser scanning microscope (Olympus IX81) with different channels. The semi-quantitative fluorescence analyses of cytoplasm and nucleus were calculated using the software *Image J* (National Institutes of Health, USA, http://imagej.nih.gov).

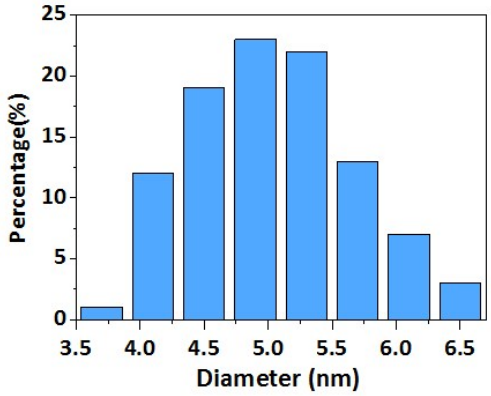


**Fig. S1** Histograms of size distribution of obtained AISe NCs with an average size of 4.9 nm (PDI = 0.042).

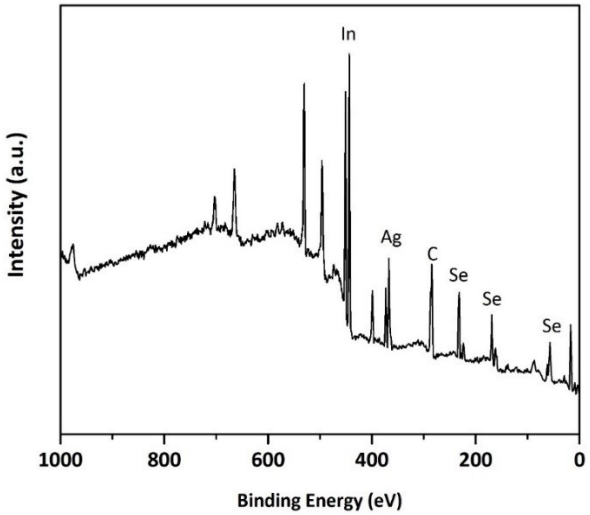


**Fig. S2** XPS survey of AISe NCs. The characteristic peaks of Ag, In and Se are clearly seen in the spectrum. The spectra were corrected by the C 1s peak at 285.0 eV.

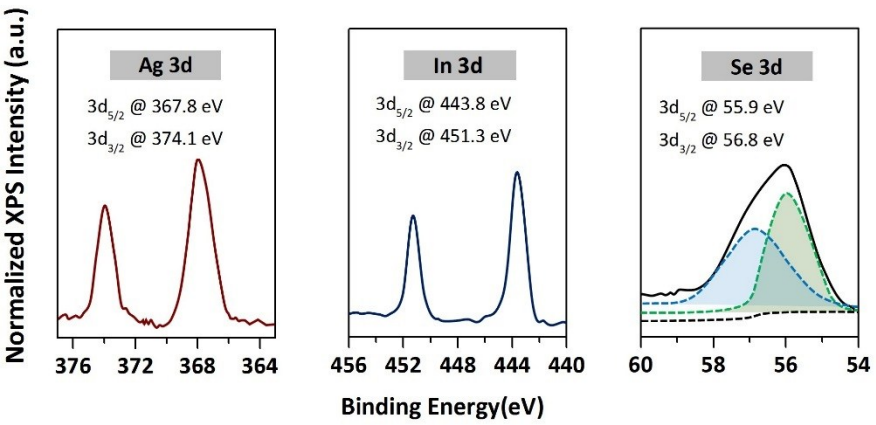


**Fig. S3** XPS narrow scans on Ag 3d, In 3d and Se 3d peaks of AISe NCs. The characteristic peaks of Ag 3d (367.8 eV for 3d5/2, 374.1 eV for 3d3/2) and In 3d (443.8 eV for 3d5/2, 451.3 eV for 3d3/2) confirm that the valence states of Ag and In are +1 and +3, respectively. The two fitted peaks of Se 3d are the splitting of spin-orbital coupling.

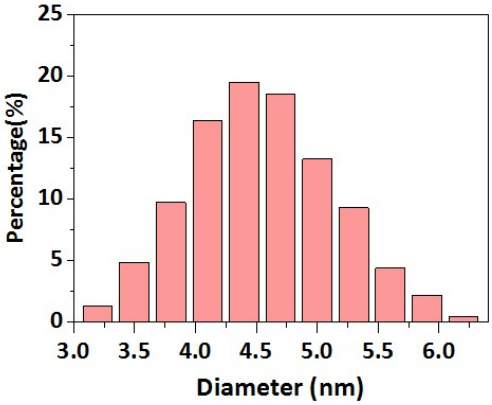


**Fig. S4** Histograms of size distribution of obtained CISe NCs with an average size of 4.5 nm (PDI = 0.039).

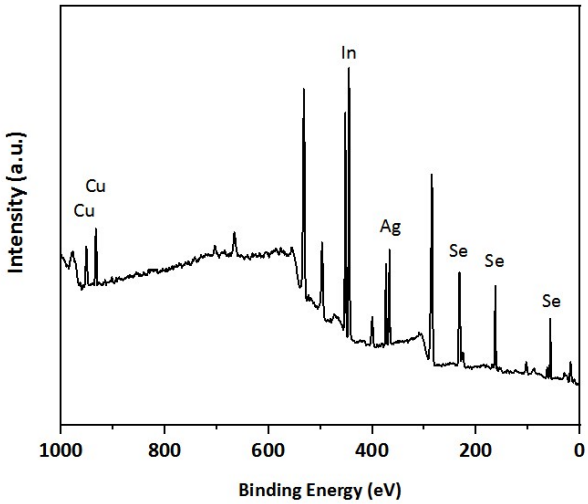


**Fig. S5** XPS survey of CAISe (*x*=0.12) NCs. The characteristic peaks of Cu, Ag, In and Se are clearly seen in the spectrum.

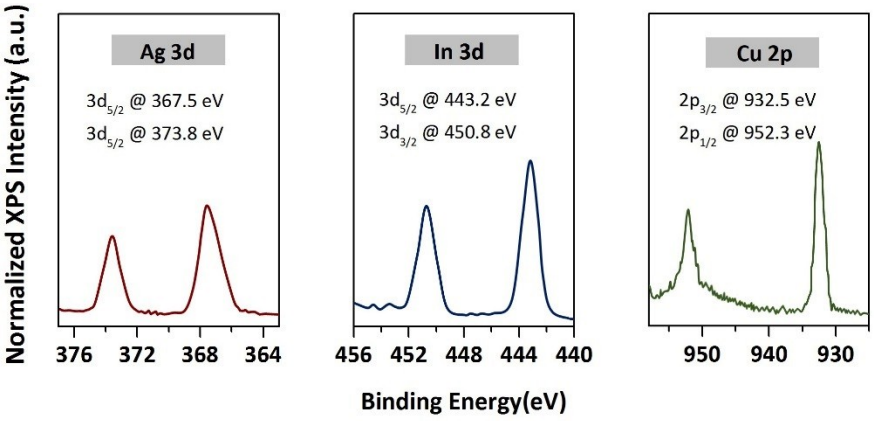


**Fig. S6** XPS narrow scans on Ag 3d, In 3d and Cu 2p peaks of CAISe (*x*=0.12) NCs (bottom panel) synthesized by CE reactions. The spectra were normalized by the intensity of Se peak.

The characteristic peaks of Cu 2p (932.5 eV for 2p3/2, 952.3 eV for 2p1/2) show the valence state of Cu is +1. It is worth noting that the Ag 3d and In 3d peaks slightly shift to lower binding energy as compared with that of the AISe sample, which may be the result of the changes in the chemical environment.

**Table S1** PL quantum yield (QY) of CAISe NCs with different *x* measured before and after DMPC modification.

| Sample | QY (before) | QY (after) |
|---|---|---|
| *x*=0 | 13.5±0.9% | 10.1±1.4% |
| *x*=0.12 | 15.6±1.1% | 12.3±1.1% |
| *x*=0.28 | 18.2±1.7% | 15.5±1.5% |
| *x*=0.43 | 20.6±2.3% | 17.6±2.8% |
| *x*=0.64 | 23.4±1.8% | 21.2±2.0% |
| *x*=0.81 | 25.9±2.5% | 23.1±1.6% |
| *x*=1 | 26.2±2.2% | 23.7±2.2% |

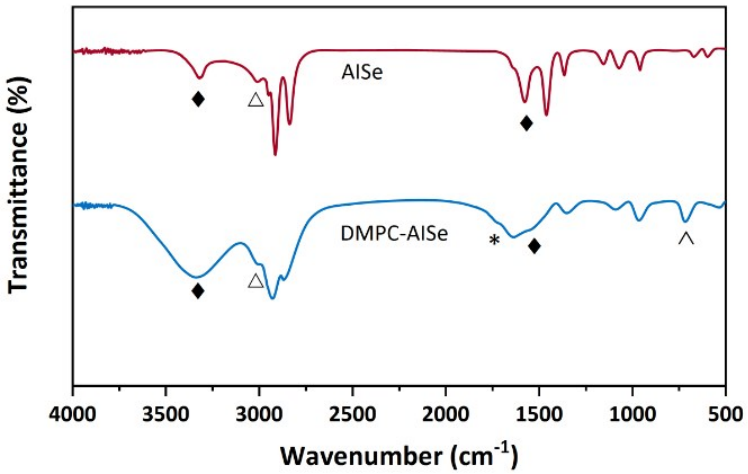


**Fig. S7** FTIR spectra of oleylamine-stabilized AISe NCs and DMPC-decorated AISe NPs. ♦: Stretching mode (~3320 $cm^{-1}$) and scissoring mode (1570 $cm^{-1}$) of $–NH_2$ from oleylamine. △:C=C stretching mode vibration (~3300 $cm^{-1}$). *: C=O stretching vibration (~1740 $cm^{-1}$) from phospholipid. ^: stretching band (~720 $cm^{-1}$) of $–CH_2$ from phospholipid chains. The FTIR spectrum of AISe shows the characteristic bands of the $–NH_2$ and C=C vibration bands of oleylamine, which is in good agreement with the reported result.[1,2] After modified with DMPC, FTIR spectrum displays the stretching vibration bands of C=O and $–CH_2$, and the characteristic bands of $–NH_2$ from oleylamine are also found, indicating that the DMPC was physically adsorbed to the oleylamine-stabilized NCs.

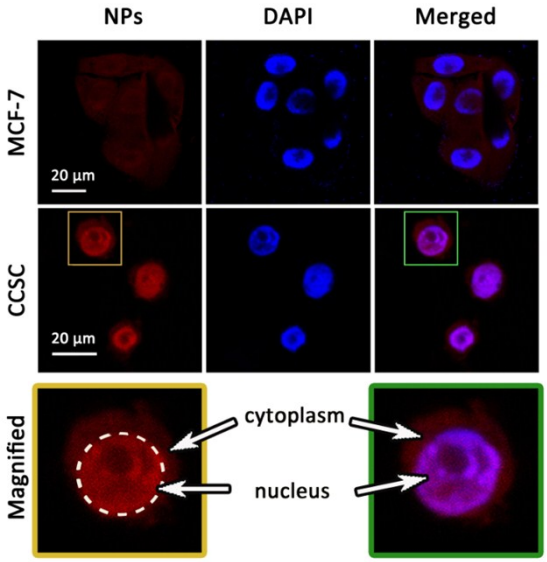


**Fig. S8** Laser confocal fluorescence images of MCF-7 cells and CCSCs recorded after incubation with DMPC-AISe NPs (corresponding to Fig. 7b in the main paper) for 5 min min and staining with DAPI.

It can be clearly observed that the nucleus of CCSCs has a higher relative intake than the cytoplasm, as compared with MCF-7 cells. From the magnified images, NPs-labeled nucleus and cytoplasm were observed in different areas. The bright purple fluorescence of the nucleus is the result of mixing the blue light of the DAPI and the strong red light of the NPs.

**References**

1. E. Muthuswamy, J. Zhao, K. Tabatabaei, M. M. Amador, M. A. Holmes, F. E. Osterloh and S. M. Kauzlarich, *Chemistry of Materials*, 2014, **26**, 2138-2146.

2. I. Kriegel, J. Rodríguez-Fernández, E. D. Como, A. A. Lutich, J. M. Szeifert and J. Feldmann, *Chemistry of Materials*, 2011, **23**, 1830-1834.